



MAKERERE UNIVERSITY

EVALUATION OF *METARHIZIUM ANISOPLIAE* FOR INTEGRATED MANAGEMENT OF TERMITE INFESTATION ON *GREVILLEA ROBUSTA* TREES IN FARMLAND

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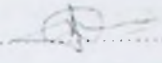
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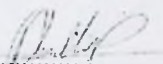
DECLARATION

I Gerald Ongodia, hereby declare that the work in this report is original and of my own effort. Where information has been obtained from other works, the authors have been duly acknowledged. I declare that this work has never been submitted to any higher institution of learning for academic accreditation.

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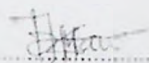
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DEDICATION

This work is dedicated to my dear children and wife to whom I am indebted;

My friends with whom I share life's moments;

My dear parents who brought me to this world and added value to my life;

And above all

The Almighty God who gives me breathe, strength and protection in all my endeavors

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ACRONYMS

ICIPE	–	International Centre of Insect Physiology and Ecology
ACDS	–	African Centre for Disaster Studies
GDP	–	Gross Domestic Product
MAAIF	–	Ministry of Agriculture Animal Industry and Fisheries
NDP	–	National Development Plan
ICRAF	–	World Agroforestry Centre
NGO	–	None Governmental Organisation
FORRI	–	Forestry Resources Research Institute
NaFORRI	–	National Forestry Resources Research Institute
NaCRRI	–	National Crops Resources Research Institute
BugizARDI	–	Buginyanya Zonal Agricultural Research and Development Institute
NARO	–	National Agricultural Research Organisation
SPGS	–	Saw log Production and Grant Scheme
HDPE	–	High Density Polyethylene
KPL	–	Kenpoly Plastics Limited
RUFORUM	–	Regional Universities Forum
SFEGS	–	School of Forestry, Environmental and Geographical Sciences
Mak	–	Makerere University, Kampala
CAES	–	College of Agriculture, Environmental and Geographical Sciences
AEZ	–	Agro Ecological Zone

ABSTRACT

This study evaluated the use *Metarhizium anisopliae* (Metschnikoff) for integrated management of pestiferous termites attacking *Grevillea robusta* trees in farm land. Aspects of the fungus assessed were persistence; optimum application rate; spread in soil and effect on non-target ground arthropods. Persistence was evaluated in farm sites applied with the fungus approximately a decade earlier whereas optimum application rate; horizontal and vertical spread in soil; and effect on non-target ground arthropods were examined within a year of fungal application to farm sites with different cropping regimes. Optimum application rate was evaluated from monthly survival of *G. robusta* tree seedlings at varying application doses of the fungal formulations. Results showed that *M. anisopliae* persisted (75.2%) in farm sites a decade after application. Survival of *G. robusta* tree seedlings improved by over 60% following application of the fungal formulations. An optimum of 66.4% seedling survival was achieved with the granular formulation at the highest dose of 2 g of the granules per seedling whereas 76.3% was the optimum survival of seedlings achieved with the oil-based formulation at a higher dose of 25 g/L per seedling. Mean spread of *M. anisopliae* in the soil was significant ($p < 0.001$) in the cropping zone nearest (0.5 – 1.5 m) to the point of fungal application under the maize + ground nuts intercrop with spread of 26.5% compared to the sole maize (11.0%) and the fallow (8.1%) whereas mean spread at the mid (1.5 – 3.0 m) and furthest (3.0 – 5.0 m) were non-significant, $p = 0.267$ and $p = 0.223$ respectively, in all the cropping regimes. Mean vertical spread of *M. anisopliae* in the shallow and deeper soil layers did not differ, $p = 0.860$, although spread was higher in the upper soil zone (16.9%) than in the lower soil zone ($11.7 \pm 14.44\%$). Non-target arthropods were not affected by *M. anisopliae* application in soil for pest termite control in farmed lands. Mean proportion of non-target arthropods not infected by *M. anisopliae* within the 3m trapping area (96.9%) was non-significantly different ($p = 0.25$) from mean proportion of non-target arthropods not infected by the fungus in the 6m area (91.9%) across all the trapping phases of 0, 12 and 28 Weeks after Application of the fungal granules. *M. anisopliae* can persist in the soil for atleast a decade with incidence levels under mixed cropping than sole cropping. Mortality of *G. robusta* trees in farmland as a result of termite attack can significantly be reduced by applying the fungus to the seedlings during planting. Fungal spread can be achieved within the surrounding points of application yet its use has no or negligible effect on other useful organisms in the soil. There is need to investigate natural occurrence of *M. anisopliae* strains in comparison with ICIPE 30 isolate thus offering a range of other options of the genus with potential for bio-control in the local agro ecologies. It is worthwhile noting that the use of the granules by farmers under intercropping conditions as an integrated approach to pest termite management is plausible.

CHAPTER 1

INTRODUCTION

1.1 Background

Agriculture is faced with counterproductive activities caused by various agents of economic importance like fungi, weeds and insect pest organisms which are responsible for annual food and crop yield reduction of 20% to 40% globally (Kachhawa, 2017). Current trends suggest that by 2050, the planet will be inhabited by 9.6 billion people with a corresponding need for increasing food production by 60% (Payne and Van Itterbeeck, 2017). This has raised fears about food availability to feed an ever-rising global population therefore calling for efforts geared towards increased production.

For the case of Africa, agriculture remains the mainstay providing food and employment to its people that make over 17% of the global population (Otieno, 2018). Development in the agricultural sector has however remained inadequate hence exacerbating the inherent problems of poverty, food insecurity leading to unsustainable growth of the continent's GDP (Salami *et al.*, 2017). Agricultural activities in Africa have, overtime, been characterized by risk averse strategies as a way to cushion the undesirable climatic and other environmental risks which including farm diversification and climate smart agriculture hence increasing the ability to cope with shocks (Waha *et al.*, 2018).

The current phenomenon of climate change even aggravates the risk factors that affect the livelihoods of households in various ways (Arslan *et al.*, 2018). Key among the factors curtailing agricultural development is the incidence of diseases and pests, among which are termites, accounting for 49% of crop yield losses in Africa, the highest on the global scene (Otieno, 2018). Country specific data on termite damage for most of the African countries is rather patchy however for Tanzania and Kenya 30% termite damage is reported while in Ethiopia, over 60% damage due to termites has been recorded whereas consultations with farmers in the humid forested areas of southwestern Nigeria revealed that up to 100% destruction by termites in maize had been observed (Maniania *et al.*, 2002).

Farmers and development partners have often indicated that the termite pest problem has often caused significant damage to field crops in the drier parts of Uganda located along within the drier cattle corridor (AFR&D, 2003). With smallholder farming continuing to play a key role in African agriculture (Salami *et al.*, 2017), the challenge of above and below ground pests

continues to manifest of which termites have become increasingly important among the below ground pests (Otieno, 2018). Known to form a large and diverse group of insects belonging to the order Isoptera with way above 2600 species belonging to 280 genera world over (Nkunika *et al.*, 2011) termites are capable of causing partial or total destruction of crops in cultivation for a longer period, exotic plants and crops including trees grown under stress conditions (Anyango *et al.*, 2020).

Most of the world's tropical and subtropical zones are faced with the termite pest problem often registering significant damage to agricultural crops, trees, rangeland and structural timber (Stansly *et al.*, 2001). In Africa, the problem is more evident in dry spells than in wet periods and more so in lowland areas than in the highlands where damage is particularly noted in crops experiencing stress conditions (Nkunika *et al.*, 2011). Attack to the plants occurs at all stages, but more so when the crop is more mature (ICIPE, 2000). For example, Lepage, 1981 reported that in the semi-arid savannah of Kenya, losses to pasture due to termites were recorded to a magnitude of 800-1500 kg ha⁻¹ of pasture per year, while in the Southern African countries, pestiferous harvester termites were registered as a serious pest of rangeland removing up to 60% and above of the standing grass biomass during dry spells (Akutse *et al.*, 2011). In Uganda, Sekamatte, 2001, reports that losses to crops and tree stands due to termite attack range from 50 to 100%.

Studies conducted by the International Centre of Insect Physiology and Ecology (ICIPE), found out that growers in some areas of Uganda and at the Kenya coast have recorded 100% field losses to improved maize varieties that apparently lack natural resistance. The problem of termites is even compounded by the recent prediction that Africa is faced with the increased risks of drought during the twenty first Century (ACDS, 2006) suggesting even greater losses due to termites attack. Among the most important termite species known to cause considerable damage in most parts of Africa are those belonging of the genus *Macrotermes* (Jirmci, 2013). Although termites are largely known to be pests of economic importance in agriculture, they also play a key role, among other soil macro organisms, in promoting vital soil ecological processes (Sileshi *et al.*, 2009). They mastermind the formation of soil biogenic structures with beneficial physical, biological and chemical properties different from those in the surrounding soil assemblages (Jouquet *et al.*, 2011). Therefore, because agriculture is the backbone to Uganda's economy employing over 80% of the labour force and accounting for more than 90% of export earnings (Mugisha-Kamatenesi *et al.*, 2008), management of pests of economic

importance including termites is a key aspect to the resource poor and small-scale farmers (Sileshi *et al.*, 2009).

Various preventative and remedial approaches are currently in use to control pestiferous termite species in the termite control industry (Chouvenc *et al.*, 2011). In the United States, estimates show that 77% of the pest control measures available on the market represent soil based termiticide applications (Chouvenc *et al.*, 2011). Owing to heavy dependence on the use of soil based insecticides and the eventual effects these might have on the environment, it is important that future termite control methods must be adapted to higher environmental standards (Chouvenc *et al.*, 2011). In Australia, for example, Milner was successful in achieving full control of *Coptotermes acinaciformis* (Froggot) by directly inoculating 3×10^{11} conidia of *M. anisopliae* isolate FI-610 into termite mounds (Wright *et al.*, 2005). Therefore fungal pathogens belonging to the genus *Metarhizium* including other groups like *Beauveria* and *Paecilomyces* have the potential for further development into commercial bio-insecticides (Ahmed *et al.*, 2009). The use of bio pesticides is considered one of the safe approaches for pest management without causing negative effects on the environment that are associated with over dependence on chemical pesticides (Dutta, 2015). Notable among non-chemical termite control approaches that has been demonstrated to have potential for bio control is the use of entomopathogenic fungus *M. anisopliae* (Hoe *et al.*, 2009). This has been shown to be effective as a biological mycoinsecticide to control termites, grasshoppers, caterpillars, aphids, red pumpkin beetle among others (Verma and Kashyap, 2019). The green muscardine fungus, *M. anisopliae* acts as a parasitoid that causes disease in various insect groups when the conidia adhere to the waxy epicuticle of the host insect eventually gaining entry to the hemocoel through a combination of complex forces and chemical processes (Verma and Kashyap, 2019).

1.2 Problem statement

M. anisopliae, has been demonstrated to be of great potential in controlling a wide range of insect pests in agriculture and forestry. Patil and Jadhav (2017), have proven that *M. anisopliae* is capable of infecting a wide range of insect pests belonging to a variety of insect orders Patil and Jadhav (2017) including those of the family Isoptera. Field trials conducted by International Centre of Insect Physiology and Ecology (ICIPE) using an isolate of *M. anisopliae* ICIPE 30 on maize in Uganda and Kenya recorded a 70% increase in maize yields in Uganda when soil was treated with *M. anisopliae* (ICIPE, 2000). The observation that termites concentrate on dead plant tissues such as wood (Debelo and Degaga, 2017), presents them as serious pests of trees in farmland. This poses a challenge to farmers who grow and

maintain indigenous or exotic trees on their farmland for various purposes depending on the needs (Humphrey *et al.*, 2019). For example, Bucagu *et al.* (2013) observe that *G. robusta* is a timber tree preferred by farmers for farm integration because it is less competitive and may be grown in close association with crops, is fast growing producing relatively large amounts of biomass, stakes and is tolerant to poor degraded soils. Farmers in Uganda ranked *G. robusta* highly among exotic trees for farm integration with agricultural crops (Kisegu *et al.*, 2019). In Kenya, farmers particularly in western Kenya also prefer *G. robusta* among other exotic species due to its faster growth rate (Humphrey *et al.*, 2019) hence providing the desired tree products within a short time. Despite all the good attributes, termites have been found to be a serious threat to *G. robusta* trees (Bucagu *et al.*, 2013) causing yield losses of 10-30% to maize in affected fields in Kenya, Uganda and Tanzania, and even higher losses of above 50% in Ethiopia and Nigeria (Otieno, 2018).

Therefore, although the findings that *M. anisopliae* has been shown to be effective in termite pest control in agriculture, there is lack of knowledge on some aspects that may be key for its effective use as part of integrated termite pest management on farms. Specifically, there is deficiency of information on its persistence in the soil over time, optimal dose and formulation to achieve high optimums for crop and tree survival, transmission in the soil from one point to another; and its effect on non-target organisms that may come in contact with the fungal propagules and may also be responsible for the transmission of the propagules from one point to another whereas they may also play an important ecological role in the ecosystem.

1.3 Justification

It is long recognized that termites are pests of economic importance because of their destructive effect on domestic wood products, construction material, tree and agricultural crops, and other goods of commercial value (Rasib and Ashraf, 2016). Their control and management has largely relied on broad spectrum and persistent organochlorine insecticides (Logan *et al.*, 1990). The continued use of these insecticides is known to result in undesirable effects on humans, animals and the environment due to their hazardous toxicity in the food chain, increased resistance in target pests, destruction of natural enemies if applied in large expanses of plantations and farmland (Remadevi *et al.*, 2010).

The stringent and legal restrictions regarding their use and effectiveness (Akutse *et al.*, 2011) as well as the high overall costs of use and the worldwide drive toward sustainable agricultural production cannot be underestimated (Guedes *et al.*, 2016). Development of environmentally

friendly biological measures for use by the resource poor rural farmers in Uganda for managing the termite pest problem is key in boosting agricultural productivity in the wake of the current era of climate change and variability.

There have been efforts directed at developing *M. anisopliae* as a bio agent for control of pests of economic importance in agriculture (Hasan, 2014) including termites in addition to good agronomic practices, physical removal of termite mounds, biological control and use of plant extracts and resistant species (Akutse *et al.*, 2011). Field trials conducted by International Centre of Insect Physiology and Ecology (ICIPE) using an isolate of *M. anisopliae* ICIPE 30 on maize in Uganda and Kenya have shown increased maize yields (Sekamatte *et al.*, 2001; Maniania *et al.*, 2002) for example, a 70% increase in maize yields was recorded in Uganda when soil was treated with *M. anisopliae* (ICIPE, 2000).

It is also reported that the effect of *M. anisopliae* ICIPE 30 on the termite population lasts more than one season (ICIPE, 2000). Unlike the commercial chemical pesticides, this product is harmless to humans and other animals and can be prepared locally at a fraction of the cost (ICIPE, 2000). Although *M. anisopliae* is known to be the best studied and demonstrated entomopathogen with great potential for use as a bio-control agent thus infecting a broad range of insect pest species (Qiu *et al.*, 2019), certain aspects of the fungus need to be further elucidated. The fact that these aspects are not fully understood offers an opportunity to effectively explore the full potential of *M. anisopliae* for use in the management of pest organisms of economic importance in agriculture and forestry.

Agriculture is a key economic activity that has increasingly faced the challenge of pests yet it is the mainstream economic activity on which a majority of rural households particularly in the tropics and subtropics depend on for their livelihoods. Key among the pests are termites that have been reported to cause significant yield losses in agriculture, for example it has been reported that maize yield losses due to termites range between 10-30% in Uganda, Kenya and Tanzania whereas in Ethiopia and Nigeria, yield losses of above 50% have been reported (Otieno, 2018). Trees in farmed landscapes have not been spared by the termite pest problem yet they play a key role in the provisioning of essential ecosystem services and products that sustain smallholder livelihoods, key among which are timber, fodder, carbon sequestration, soil fertility enhancement (Humphrey *et al.*, 2019). Studies on *M. anisopliae* entomopathogen for termite control have in the past majorly focussed on laboratory experiments as well as field experiments for agricultural crops while ignoring trees which are an important component of farming. With the focus on exotic tree species that are thought to provide the desired tree

products within a short time, *G. robusta* is such a tree species that has gained prominence among farmers (Nath *et al.*, 2016), it is worthwhile investing in efforts to increase tree survival in landscapes.

1.4 Overall objective

To evaluate the potential of *M. anisopliae* for integrated management of termite pests on *G. robusta* on-farms.

1.4.1 Specific objectives

1. To assess persistence of *M. anisopliae* in the soil over time at farm sites treated with the fungus
2. To determine optimum doses of granular and oil-based formulations of *M. anisopliae* for management of termite infestation on *G. robusta* seedlings.
3. To assess horizontal and vertical spread of *M. anisopliae* in soil under different cropping regimes.
4. To assess the effect of *M. anisopliae* on non-target ground arthropods.

1.4.2 Hypotheses

1. There is no difference in *M. anisopliae* persistence in soil over time at farm sites applied with the fungus against termites.
2. There is no difference in effectiveness of the different doses of *M. anisopliae* applied against termite attack on *G. robusta* tree seedlings.
3. There is no difference in horizontal and vertical spread of *M. anisopliae* in soil under different cropping regimes.
4. There is no difference in the effect of *M. anisopliae* on non-target ground arthropods exposed to the fungus over time.

CHAPTER 2

LITERATURE REVIEW

2.1 General

World over, social insects have been considered the most successful fauna at colonizing new environments. Among the soil borne macro fauna that form a large percentage of the most successful social insects are termites (Kumar and Pardeshi, 2011). Termites form part and parcel of the ecosystem and have evolved for over 300 million years.

In the semi-arid tropics, termites are considered the most important soil fauna often found in a wide range of terrestrial environments in the tropical, sub-tropical and temperate zones of the world. Termites abundantly occur in tropical ecosystems and are often found acting on dead plant material in soil and in so doing, play an important role in nutrients cycling and soil fertilization (Miranda *et al.*, 2004). They participate in fragmentation of the plant necromass preparing it for mineralization through fungal and bacterial action subsequently transforming it into inorganic nutrient compounds ready for plant absorption. Through these processes, termites have a marked effect on the structure and properties of tropical soils. Several of termite species move organic matter from the surface to deeper soil layers thus improving and maintaining soil porosity and aeration (Miranda *et al.*, 2004). Whereas all these are beneficial in the ecosystem, numerous species of termites are destructive and are a major threat to agriculture in tropical countries (Wardell, 1987). Some termite species are able to kill healthy tree crops, and therefore, have the potential to cause much greater losses (Debelo and Degaga, 2017).

The African continent is, to a large extent, considered the richest in termite diversity (Sileshi *et al.*, 2009). In eastern and southern Africa, the sub family Macrotermitinae has been known to reach their highest concentrations and have been identified as the most serious pests in agriculture and forestry, (Kumar and Pardeshi, 2011).

In agricultural systems, termites have been reported to cause harm to many crops, like maize, yam, cassava, peanut, rice, and cotton (Miranda *et al.*, 2004) whereas in forestry, many termite species have been reported to cause severe economic damage to timber and its products (Remadevi *et al.*, 2010). In cases where termites do not cause total death of the tree, they may cause damage to the bole, for example, by consuming the heartwood and, thereby, hollowing

the trunk thus reducing the quality and value of the tree as a source of timber (SPGS, 2010. No 27). Conversely it is known that termites may greatly impact on soil properties and soil formation. A high concentration of termite mounds and galleries plays an important role in improving soil porosity and aeration, permeability and water holding capacity and consequently, improving soil primary productivity (Mando and Van Rheenen, 1998). Termites are also important in nutrient recycling and by so doing amend soil chemical properties by mixing soil from different layers. It is common practice among farmers in some African to deliberately find land with a dense network of large termitaria or areas where termites are often found foraging for reasons of soil fertility or availability of soil moisture and in addition, these areas are considered to influence plant successions, patterns and fallow dynamics (Mando and Van Rheenen, 1998).

2.2 Termites

Termites are communal insects with well-ordered groups living in terrestrial environments thereby making a significant impact in the ecosystem through the role they play in decomposition activities (James, 2019). Termites are also known for the damage they cause to woody plant parts hence negatively impacting on plant production (James, 2019).

Although, in ecological terms, termites are important in the re-utilization of nutrients, the damage that they cause to crops, pastures and trees through their activities are a major deterrent to ecosystem productivity (Mali *et al.*, 2018).

Worldwide, at least 43 termite species are known to be of nutritional value as food to humans and livestock, this being probably most popular in Africa (Govorushko, 2019). Krishna and Weesner, 1970 recognised that termite families known to occur in tropical forests and grassland savannahs world over include *Mastotermitidae*, *Kalotermitidae*, *Hodotermitidae*, *Termopsidae*, *Rhinotermitidae*, *Seritermitidae* and *Termitidae*. The majority of termite genera considered of economic significance in agriculture and forestry are those belonging to *Macrotermes*, *Allodotermites*, *Amitermes*, *Pseudacanthotermes*, *Odontotermes*, *Ancistrotermes*, *Trinervitermes*, *Hodotermes* and *Microtermes* genera (Materu *et al.*, 2013). The magnitude of termite damage to a tree and loss caused are a function of the geographic location in question (Debelo and Degaga, 2017).

2.3 Termite foraging

It is often known that termite feeding activities tend to concentrate on dead cellulose material and soil rich in organic matter thus causing serious loss in suitable crop land environments.

Among all the recognized termite species, only 300 species are known to be pestiferous in agriculture (Materu *et al.*, 2013). Harris, 1955; Lee and Wood, 1971 observed that the pattern of termite damage to plants varies with species, climate and area, however it is widely assumed that termites rarely damage indigenous trees. This is said to be so because it is affirmed that indigenous trees have developed defense systems against indigenous termites (Gould *et al.*, 1993). In a number of studies conducted in Malaysia, it is highlighted that there are high severity and incidence levels of termite attack in conifer plantations for which *Coptotermes curvignathus* Holmgren, has been identified as a key pest species of plantation forests, killing and damaging trees of any age (Jasmi and Ahmad, 2011). There are a number of constraints that the problem of termite infestation can have including agronomic, economic, or social constraints. The agronomic constraint includes the functioning of termites as pests and ecosystem engineers; whereas, the economic constraints involve the destructive tendencies of termites as a result of their feeding activities on plants and woody cellulosic material thus causing economic hardship to individual producers (Maayiem *et al.*, 2012). Despite the fact that termites play a major role in improving the soil fertility, there has been a growing concern of termite pest problem to agricultural crops, forest trees and grasslands (Materu *et al.*, 2013) especially in Africa where the economy is dependent upon subsistence agriculture, for example farmers in Rufiji district previously indicated that termite pest problems tended to increase after the forest was opened for food and cash crops cultivation, the main crops cultivated being maize, cassava, rice, cashew and coconut (Materu *et al.*, 2013). There is an ever-growing interest in sustainable agriculture and food security on the African continent. In light of this, there is a call to develop more balanced approaches to termite control that will minimise serious ecological damage that will consequently result in loss of ecosystem services that termites provide (Maayiem *et al.*, 2012).

2.4 The termite predicament

World over, termite management is approximated to take largest portion worth 8 billion US dollars in the global pest control industry. Studies show that the economic damage caused by termites sums to billions of dollars in the United States, Australia and Japan. Because of the rather evident problem that termites present on food production as well as industrial crops, it is paramount that information on population dynamics, outbreaks, damage incidences as well as available management interventions for termite infestation is essential in informing producers in termite susceptible zones. Facts on economic damage for some African countries is available, for example, in Kenya and Tanzania, Gitonga *et al.*, 1995 has recorded losses of up to 30% due to termites while in Ethiopia 60% termite damage has been recorded by Wood,

1986 (Maayiem *et al.*, 2012). In south western Nigeria, personal interviews conducted by Umeh and Ivbijara, 1997, with famers have disclosed that up to 100% damage on maize by termites has been realised (Maayiem *et al.*, 2012).

2.5 Termite control

In the recent past, termite control was purely dependent upon chemical insecticides especially of the synthetic types. Satisfactory termite control in agro-ecosystems was mainly dependent upon persistent organochlorine (OC) insecticides (Ahmed *et al.*, 2006). The introduction of innovative chemical pesticides, saw this crisis being resolved to a great extent, however, over dependence in chemical use has raised serious environmental concerns thus calling for efforts aimed at developing safe alternative approaches (Kachhawa, 2017). Control of termites by chemical means both in plantations and farms is rather expensive and requires skilled labour which in addition may be ineffective in all cases (Maayiem *et al.*, 2012). Termiticides applied in excessive amounts are likely to cause environmental pollution. Dennis, 1981, reported that termiticides may also cause the death of non-target organisms thus necessitating the ban of some chemical control measures (Maayiem *et al.*, 2012) and withdrawal of the persistent organochlorines from the world markets. Today, there are various safe and yet simple practices of termite management are being practiced some of which include cultural and biological control, queen removal, use of resistant plants, natural products, physical barriers and baiting though insecticides still play a key role in termite control.

2.5.1 Physical methods

These approaches may involve breaking the termite mounds and removing the queen and the king (Otieno, 2018). This method is labour intensive, because termites construct mounds from very hard building material and the mounds maybe numerous and of considerable dimensions. The success of this measure is highly dependent upon complete elimination of the queen, who may be hidden deep inside and is not easy to locate. The recommendation to mechanically destroy termite mounds can be made if they appear close to structures like houses and storage granaries (Kumari *et al.*, 2013). Another approach involves the use of physical barriers that take the form of materials like sand or gravel aggregates, metal mesh or sheeting that prevent termite entry through the impenetrable material thus acting as a physical or mechanical barrier to prevent termite penetration and damage to structures (Kumari *et al.*, 2013). These involve introducing strong bar magnets in the soil within the proximity of a new termite mound effectively preventing a mound from growing. The magnets perturb those termite species

which build their mounds along magnetic lines in the north-south direction (Kumari *et al.*, 2013).

2.5.2 Cultural methods

Termite diet is largely compost dead plant material thus it is considered that termite attack is often associated to soils with low content of organic matter (Anyango *et al.*, 2020). This is because such soils contain inadequate food to sustain termites and therefore the only option they may have is to feed on living plant material. Addition of compost or well decomposed manure to the soil and growing green manures helps to boost the organic matter content in the soil. Prevention of termite attack on the target crop can be achieved by ploughing green manure crops into the soil because the moisture and the organic matter content in the green manure act as termite attractants (Kumari *et al.*, 2013). Incorporating castor press cake into the soil controls termite infestation and this may be achieved by adding it into the opened furrow before the crop is sown. By making ridges and shaping the soil into a ridge form along the contours, termite colonies are destroyed and/or exposed to predators such as birds (Sahayaraj, 2018).

Diseased plants or plants suffering from water stress are often distressed and therefore more susceptible to termite attack than healthy plants. It is therefore good practice to keep healthy and well-watered plants (Anyango *et al.*, 2020). Sowing of seeds especially in dry areas is recommended at the beginning of the wet season. This allows the root system of the plants to properly establish in the soil and remain healthy. During the process of transplanting and pruning, great care must be taken to avoid injuries to leaves and roots as termites may gain access to the plants these scar tissues (Tilahun, 2018). Removal of termite-infested vegetation, for example trees, and dead trees prior to commencement of the planting season usually at the on-set of the rains may help to prevent swarming from the areas of infestation thus helping in eradicating considerable termite populations in such areas (Paul *et al.*, 2018).

Crop rotation plays an important function in abating termite attack, preventing pest and disease buildup and also helps the soil recover its fertility. According to the HDRA Report, 2001, farmers in some parts of India, castor (*Ricinus communis*) crop in severely termite infested fields and by doing so they found that the termite infestation was substantially reduced in the next cropping season. Regular irrigation of farmlands has been found to reduce termite activities and consequently reduced damage on crops due to termites (Kumari *et al.*, 2013).

Termites often cause severe damage to crops like maize during maturation towards harvesting time than earlier during the season (Loko *et al.*, 2019). It is therefore recommended to carry out prompt harvesting.

Generally, indigenous crops exhibit more resistance to termites than exotic crops (Samaila, 2019). Similarly, annual crops are prone to termite attack towards harvest time whereas perennial crops are severely attacked during dry seasons or during early stages of growth (Nyamwasa *et al.*, 2018). It may be worthwhile establishing small plantations in the field preceding larger scale plantations. This helps to check if, under the local conditions, is attacked by termites. In addition, some practitioners of organic agriculture spread dry leaves, farm litter and other waste material in the field and spray water on it (Loko *et al.*, 2019). By so doing, they are able to divert the attention of termites to these materials and by this; they are able to prevent termites from spreading and consequently attacking the crops.

Termite control by biological measures is generally complex because termites are social insects in nature and often reside in securely enclosed environments that protect them against most enemies. Some of the biological termite control measures employ bio-entomopathogens like fungi, nematodes and bacteria (Litwin *et al.*, 2020).

2.5.3 Entomopathogenic fungi

Entomopathogens have successfully been used in integrated pest management in many control programmes. Entomopathogenic fungi are thought to achieve their killing effect on the host insect through physiological starvation by consuming all the insects' nutritional reserves when in an infective spore stage. The fungal germ tube so formed when the spores germinate and penetrates the host insect's cuticle thus invading the insect hemocoel (Abonyo *et al.*, 2016). If conditions are favourable, the fungus multiplies and grows out of the insect forming conidiophores and sporulate (Grund and Hirsch, 2010). Because entomopathogens have a relatively narrow host range, they are considered for use as suitable biological pesticides (Grund and Hirsch, 2010). Fungal isolates have the capacity to withstand unfavourable conditions during which the spores are dormant stage. This tendency allows them to survive through periods when hosts are not present (Grund and Hirsch, 2010).

Many insects predate on termites as a source of protein namely spiders, beetles, flies, wasps and more so ants. Among other predators are frogs, reptiles, birds and mammals such as bats and monkeys. Predation on termites by other groups occurs during mating flights, when large

numbers of winged individuals are susceptible to predation, mainly by birds (Tuma *et al.*, 2020). Encouraging such predator wild life may help to control termite numbers hence reducing the effect of pestiferous termites (Kumari *et al.*, 2013). Experiences drawn from other parts of the globe especially the third world indicate that interplanting certain weed plants with crops is an environmentally friendly approach for the management of emerging pests of economic importance in agriculture e.g. termites (Samaila, 2019). The weed plants are habitat to other organisms like ants that predate on the pest thus putting the pest populations on check (Latifian *et al.*, 2018).

Certain plant parts and extracts possess a range of pesticidal and insecticidal properties that cause repellency, antifeedancy, growth regulation and toxicity to agricultural pests. These extracts may be used as concoctions which when mixed and applied to areas of infestation can be used to control pestiferous insects including termites (Kumari *et al.*, 2013). Kalyani, Kalaynrao and Shikha, 2013, have demonstrated that leafy extracts of following plants can be used to control termite: *Azadirachta indica* (Neem), *Allium sativum* (Garlic), *Ocimum canum* (Green basil), *Carica papaya* (Papaya), *Jatropha curcas* (Ratanjot), *Argemone mexicana* (Mexican poppy), *Camellia sinensis* (Tea), *Santalum album* (Sandalwood) *Tagetes minuta* (Mexican marigold), *Tectona grandis* (Teak), *Tagetes erecta* (Marigold), *Ricinus communis* (Castor), *Calotropis gigantean* (Madar), *Cannabis sativus* (Hemp), *Curcuma amada* (Mango Ginger), *Datura alba* (Datura), *Eucalyptus globules* (Blue gum), *Lantana camara* (Lantana), *Musa paradisiacal* (Banana), *Pongamia pinnata* (Poongam), *Parthenium hysterophorus* (Carrot grass). In addition to extracts from neem, farmers in other parts of Asia and Africa have used wild tobacco, dried chilly, Calotropis and wood ash for controlling and repelling termites (Sohail *et. al*, 2004). Baiting techniques for termite control have, in recent times been developed as a major alternative to protect structures, timber and crops against termite damage. Baiting takes advantage of the inherent termite behaviors of interdependence, trophallaxis, mutual grooming and cannibalism to spread the bait toxicant throughout the colony thus resulting in loss of the colony and the entire termite population (Dhang, 2011).

Su, 1994 and Su *et al.*, 1995 have reported success in baiting techniques using the chitin inhibitor hexaflumuron (Mugisha-Kamatenesi *et al.*, 2008). Hexaflumuron based baits and other toxicants are now being used for control of termites on structures although none are currently labeled for agricultural use in the US (Mugisha-Kamatenesi *et al.*, 2008).

CHAPTER 3

MATERIALS AND METHODS

3.1 Description of study area

This study was carried out in the districts of Wakiso, Mayuge, Luuka and Namutumba. Wakiso, Mayuge and Luuka districts are located in the Lake Victoria Crescent Agro Ecological Zone (AEZ) whereas Namutumba is in South East L. Kyoga Flood Plain (AgriFoSe2030, 2018). Wakiso district is found in the Central Region of Uganda partly encircling Kampala, Uganda's capital city. The district borders Mukono to the East, Mubende and Mpigi to the West, Luwero to the North and Kalangala to the South. Wakiso has a population of over 562,609 people, of which 282,887 are female and 277,722 are male. The economic activity in Wakiso is agriculture based with the growing of sweet potatoes, beans, cassava, groundnuts, Irish potatoes and soya beans in addition to fishing on Lake Victoria; and poultry feeds. Cash crops grown are; coffee and cotton. Fruits and Vegetables like tomatoes, onions and cabbage are also widely grown (GOVERNMENT, 2015).

Mayuge district is found in eastern Uganda and until the year 2000, was part of the greater Iganga district. Mayuge is bordered by the current Iganga district to the north, Bugiri district to the northeast, Namayingo district to east, the Republic of Tanzania to the south and Jinja district to the west (<http://www.ugandatraveltguide.com>). A significant proportion of about 77% of the district surface area is open water of Lake Victoria and 10% is protected forest reserve. There are also numerous islands in Mayuge which are occupied by fishermen. It is estimated that Mayuge's human population stands at 461,200 people out of whom 51.2% are female and 48.8% are male. The fertile soils and favourable climate make Mayuge have a great agricultural potential, however with the open water and forest reserves occupying an estimate of 87% of the surface area, there is significant amount of pressure exerted on the natural environment by the increasing population. Agriculture in the district is therefore subsistence with the growing of mainly maize, cassava, groundnuts, beans, sweet potatoes, cotton and coffee (Kyarikunda *et al.*, 2017). Fishing is another key economic activity in the district contributing a significant amount of revenue to the district.

Luuka district is located in eastern Uganda and was carved out of the greater Iganga district in 2010. Luuka District is bordered by Buyende district to the north, Kaliro district to the

northeast, Iganga district to the southeast, Mayuge district to the south, Jinja district to the southwest and Kamuli district to the northwest.

Namutumba district is located in South-eastern Uganda, approximately 125km from Kampala City and became a district in 2006, created from the then Iganga district that originally also covered the current Bugiri and Mayuge districts. It borders Iganga district to the south, Bugiri to the southeast, Kaliro and Pallisa to the north, and Butaleja to the east.

The district covers a total area of 692.83 square kilometres and consists of one county with six sub-counties and one town council. The district is poor economically and it is estimated that the number of poor individuals stands at 78,697 out of a population of 118,807. Household welfare is poor because of the high poverty level and households depend mainly on subsistence farming for livelihood (OPM, 2016).

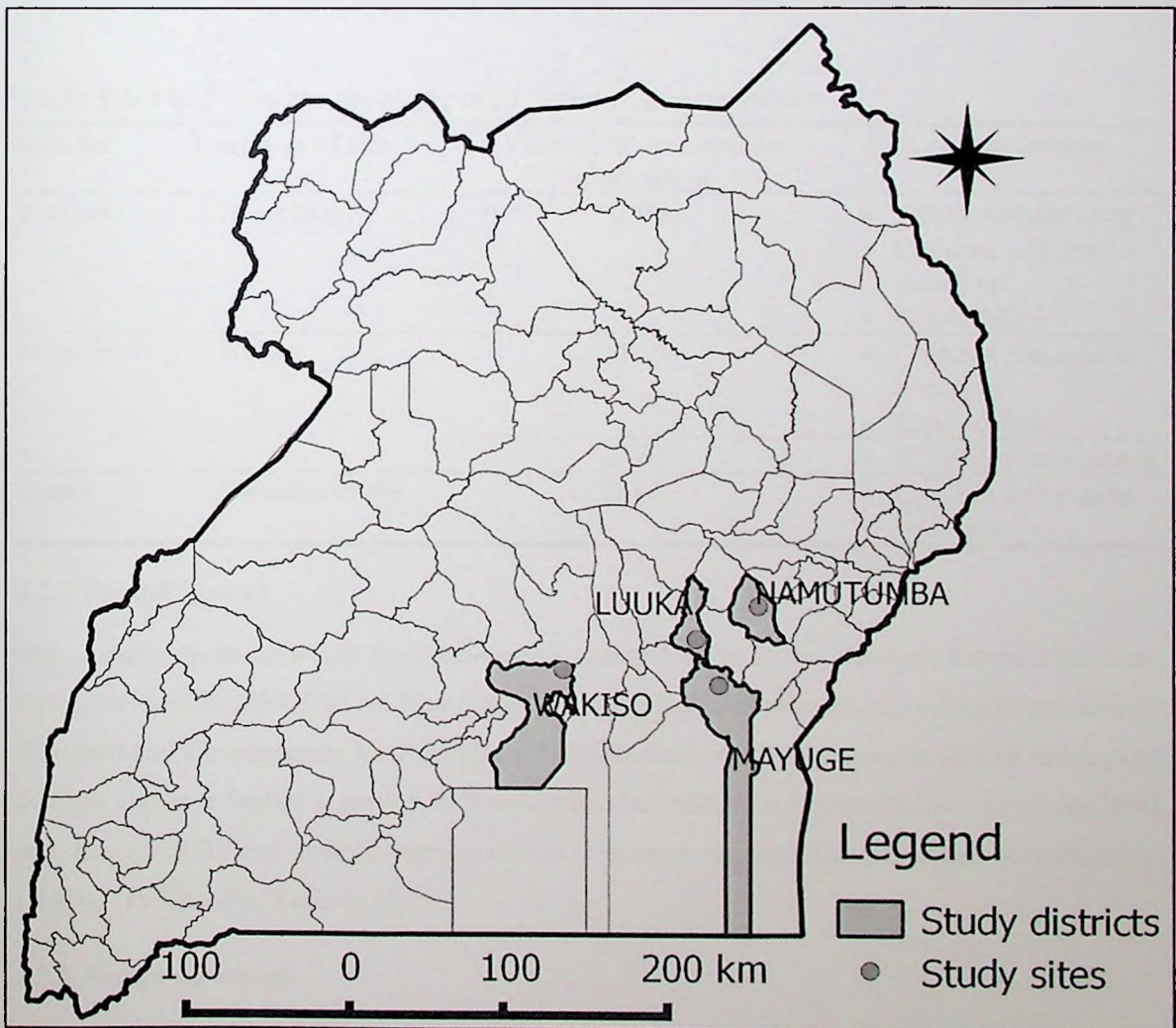


Figure 3.1: Map of Uganda showing *M. anisopliae* study sites

3.2 Persistence of *M. anisopliae* in soil

3.2.1 Study sites

Study sites for assessing persistence of *M. anisopliae* in the soil (Table 3.1) were selected based on earlier experiments conducted to control pestiferous termites attacking and causing damage to agricultural crops and trees on farm land (Sekamatte, 2000). In Wakiso district, *M. anisopliae* was applied to on-station research plots at the National Crops Resource Research Institute (NaCRRI/NARO) in Namulonge to control termites attacking maize and cassava crops (Sekamatte, 2000). In Mayuge, *M. anisopliae* was applied against termite attack on cassava, maize and *G. robusta* (Sekamatte, 2000) trees at Ikulwe on-station research gardens under Buginyanya Zonal Agricultural Research and Development Institute (BugiZARDI), whereas at Namukubembe village in Luuka district, the fungus was applied in farmers' fields to protect against attack by termites on maize and cassava crops (Sekamatte, 2000).

Table 3.1: Field sites for the study of *M. anisopliae* persistence

District	Location of site	Year of <i>M. anisopliae</i> application	Site characteristic
Wakiso	Namulonge	2000	• Tilled without crop • Cassava and maize intercrop • Grass fallow
Mayuge	Ikulwe	2000	• Cassava and maize intercrop • Grass fallow
		2003	• <i>G. robusta</i> tree stand
Luuka	Namukubembe	2000	• Cassava and maize intercrop

3.2.2 Sampling sites

Soil samples were collected from on-station research sites at the National Crops Resources Research Institute (NaCRRI) at Namulonge in Wakiso district; Buginyanya Zonal Agricultural Research and Development Institute (BugiZARDI) field research station at Ikulwe in Mayuge district; and at a farmer's garden in Namukubembe village in Luuka district. Both NaCRRI and BugiZARDI are establishments of the National Agricultural Research Organisation (NARO) in Uganda (Table 3.1).

3.2.3 Sampling design

Square plots of 5m x 5m were established at the sites where *M. anisopliae* was applied to the soil to control termite damage on cassava and maize crops; and *G. robusta* trees. Soil samples

were taken from four randomly selected spots in each plot using a soil corer of 7.5 cm diameter and 5cm height by inserting the corer into the soil to a depth of 10 cm (Pérez-González *et al.*, 2014). At a minimum separation of 15 m from *M. anisopliae* application sites, other square plots of 5 m x 5 m were established and control soil samples taken following the same procedure as described above. Before collection of the control soil samples, the soil corer was first washed with clean water and surface sterilised by wiping with cotton wool soaked in 96% ethanol. All the soil samples collected from the fungus applied and control plots were separately loosened into 30-micron non-perforated H.D.P.E. polythene bags (KPL, Nairobi) and placed in a cooler box. The cooler box with its soil contents was transported to Makerere University, School of Forestry, Environmental and Geographical Sciences (SFECS) pathology laboratory. In the laboratory, the soil samples were aggregated in respect of their collection sites to make composites. The soil composites were thoroughly mixed by hand while breaking any soil lumps to make a homogenous mixture for further use.

3.2.4 Exposure of termite specimens to soil samples

One portion of approximately 500 g was obtained from each composite soil sample and placed in 2 L plastic buckets (Kenpoly) surface sterilized by wiping with cotton wool soaked in 96% ethanol. Twelve live and active soldier termite individuals of the *Macrotermes bellicosus* species collected from termitaria around the National Forestry Resources Research Institute (NaFORRI) in Kifu, Mukono, were placed in each bucket containing the soil portions. The specimens were allowed to freely move in the soil at room temperature for about 15 hours (James *et al.*, 2020). After this time, ten specimens were removed from each bucket and transferred to sterile petri dishes lined with filter paper moistened with distilled water. Moistening of the filter paper was done using a hand spray pump. The petri dishes containing the termite specimens were incubated at room temperature on a lamina floor surface sterilized with 96% ethanol (Cabrera-Mora *et al.*, 2019). The petri dishes with their contents were inspected after every two days and any termite cadavers that showed a green growth of the mescardine disease characteristic of *M. anisopliae* fungal spores were removed from the petri dishes using a pair of sterilised forceps, counted and recorded. Periodic moistening was done at the time of inspection to ensure that the filter paper remained moist and allow sporulation of the cadavers (Murigu *et al.*, 2016). Inspection and removal of diseased termite cadavers was carried for 24 days.

3.3 Optimum application dose of granular and oil-based *M. anisopliae* formulations

At Magada and Bulagazi villages in Namutumba district, farm sites were selected for determination of optimum application rate of the granular and oil-based spore formulations of *M. anisopliae* for controlling termite infestations on *G. robusta* seedlings. This was based on an outcome from a needs assessment conducted during the implementation of the Agroforestry Research and Development project between 2002–2006 by the then Forestry Resources Research Institute (FORRI). It emerged from the communities at a needs assessment exercise that termites were a major pest attacking and causing serious damage and losses to *G. robusta* trees, a suitable exotic spp for farm integration with maize and ground nuts, key staple crops for farming communities in Busoga sub region (AFR&D, 2003).

Household farmers from Magada and Bulagazi villages in Magada sub county, Namutumba district, were mobilized for a meeting to sensitise them about the purpose of the study. The farmers who willingly volunteered to participate in the study were requested to offer the required land in their farms to set up the experimental plots to be planted with *G. robusta* seedlings. The areas of the farm were those where the farmers observed high termite infestation. The participating farmers were also required to weed and protect the seedlings from damage by stray animals. Five farmers were selected and willingly offered their land to host and participate in the experiment; three from Magada and two from Bulagazi villages, all in Magada sub county. Termite infestation on farms is rather variable from one farm to another hence the replication to improve accuracy of the data. Each of the experimental farms was considered a block with each of the villages hosting three blocks thus resulting in a total of six experimental blocks.

3.3.1 Land preparation, plot set-up and planting of *G. robusta* seedlings

Initial land preparation at each farm site was done using an ox drawn plough followed by secondary tilling using a hand hoe in preparation for planting of *G. robusta* seedlings. Six plots, corresponding to the treatments, each measuring 8 m x 6 m with a separation of 2 m between the plots were lined out at each site (**Figure 3.2**). The plots were each planted with eight months old *G. robusta* tree seedlings spaced at 2 m between seedlings in a row with a separation of 2 m between rows. Every plot accommodated 20 seedlings resulting in 120 seedlings per farm site and each site of six plots was considered a block. The seedlings were acquired from the general tree nursery at the National Forestry Resources Research Institute (NaFORRI) in Kifu Mukono, a reputable source of tree planting germplasm.

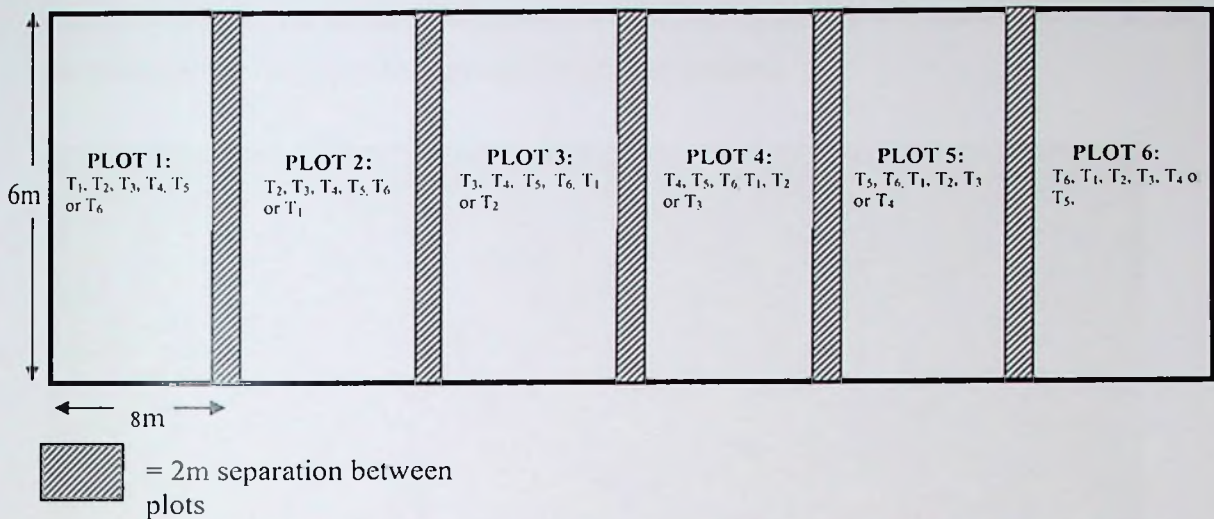


Figure 3.2: Diagram of the plot lay out for determining optimum application rates of granular and oil-based spore formulations of *M. anisopliae*

3.3.2 Formulations of *M. anisopliae* treatments

M. anisopliae, ICIPE 30 isolate, was acquired from the International Centre of Insect Physiology and Ecology (ICIPE) in Nairobi, Kenya. The acquired isolate had a concentration of 1×10^9 conidia per gram and was made into two formulations i.e. granular and oil-based spore formulations. The granular formulation had *M. anisopliae* spores embedded on rice grains (Abonyo *et al.*, 2016) as a carrier material (Figure 3.3). The *M. anisopliae* spore coated granules were weighed into 60 g, 120 g and 240 g quantities using an electric balance at SFEGS pathology laboratory. The weighed granules were separately put in H.D.P.E polyethylene bags, wrapped in aluminium foil and packed in paper boxes. Similarly, 125 g and 250 g of *M. anisopliae* pure spores were weighed at SFEGS pathology laboratory and the weighed spores treated as described above for the granules. These procedures were undertaken for safe handling and transportation of the spore materials to the field sites. After arrival at the field sites, the oil-based formulation of *M. anisopliae* was made by separately mixing 125 g and 250 g of pure spores in one litre of vegetable cooking oil and diluting the mixtures (Mnyone *et al.*, 2009) with 10 litres of water obtained from a borehole. The separate mixtures were thoroughly stirred by hand in plastic buckets (Kenpoly) to obtain homogenous solutions (Figure 3.4) with respective standard concentrations of 12.5 g/L and 25 g/L. Hence treatments per seedling in

respective plots for the granular formulation were 0.5 g, 1 g and 2 g whereas treatments for the oil-based formulation were 12.5 g/L and 25 g/L per seedling.



Figure 3.3: Granular formulation of *M. anisopliae*



Figure 3.4: Oil-based formulation of *M. anisopliae*

3.3.3 Application of the treatments to *G. robusta* seedlings

Treatments of the granular formulation (0.5 g, 1 g and 2 g) were randomly allocated to the plots and each applied to *G. robusta* seedlings in respective plots in each block by hand-

sprinkling the granules to the soil at the base of each seedling after planting (**Figure 3.5**). A thin layer of soil was lightly spread over the granules to avoid possible carrying away by wind. Treatments of the oil-based spore formulation (12.5 g/L and 25 g/L) were applied by spraying (Abonyo *et al.*, 2016) the solutions to the soil at the base of each *G. robusta* seedling in the respective plots using a one-litre capacity hand sprayer (**Figure 3.6**). To achieve adequate wetting, each seedling was sprayed with approximately 83.3 ml of the solution. The treated seedlings were assessed for termite damage and subsequent death every four weeks after application of fungal treatments. The assessments were done for a period of 44 weeks. Seedlings that were found to have been damaged and dead in every plot as a result of termite attack were counted and recorded.

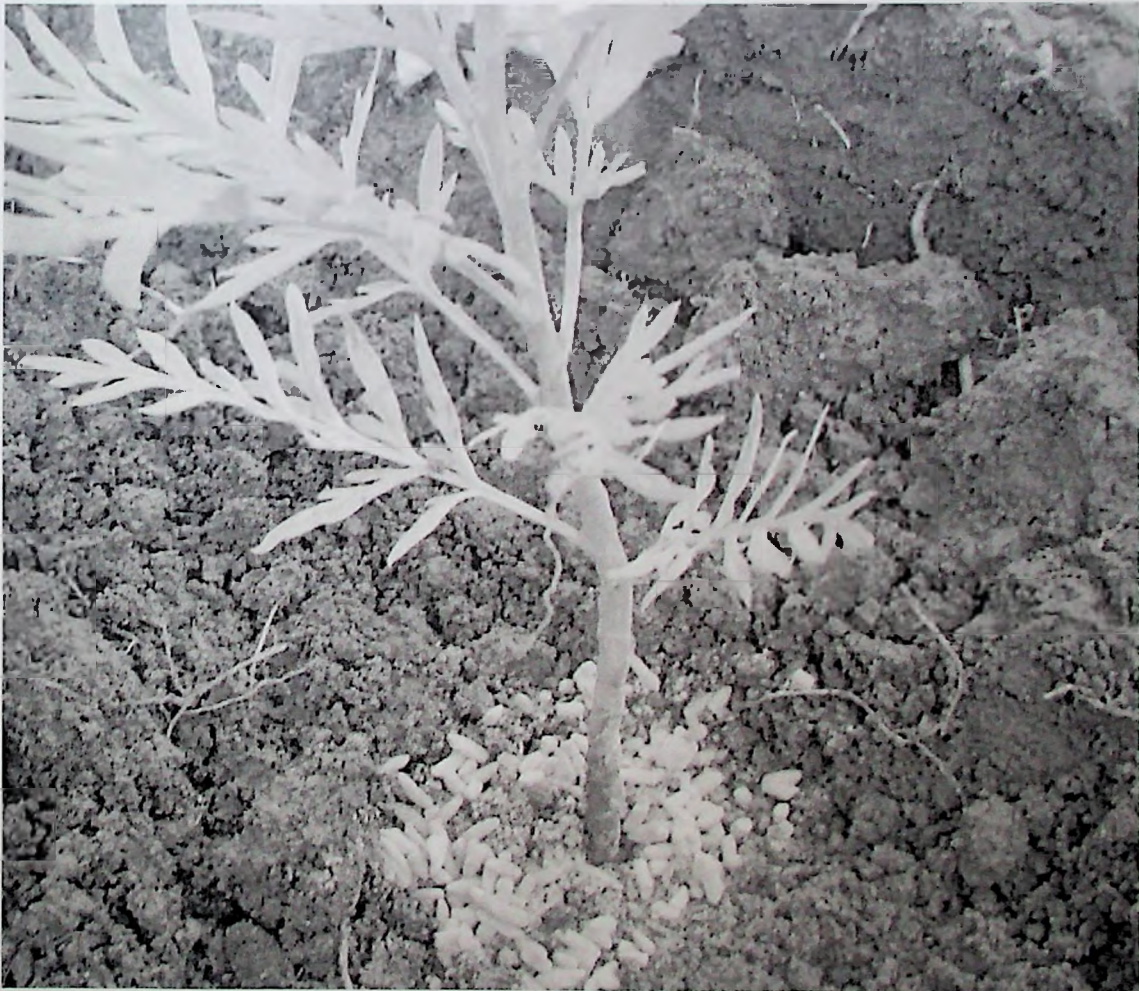


Figure 3.5: Granules of *M. anisopliae* sprinkled around a planted *G. robusta* seedling

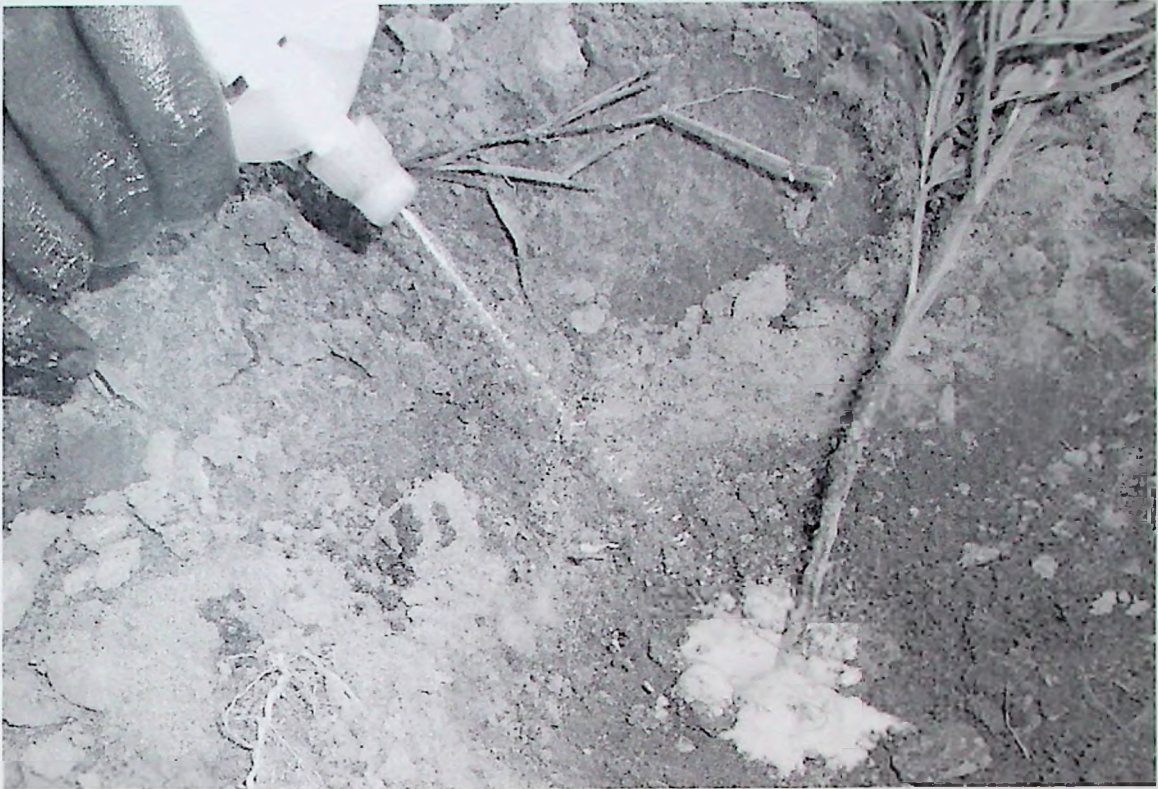


Figure 3.6 An oil-based solution of *M. anisopliae* formulation sprayed around a planted *G. robusta* seedling

3.4 *Metarhizium anisopliae* spread in soil under different cropping regimes

Three plots, corresponding to the planting regimes (maize + ground nuts; sole maize; and fallow), each measuring 15 m x 8 m at a separation of 2 m between them were established on farms alongside each other (Figure 3.6). In this study, *G. robusta* is intercropped with maize and groundnuts because it is the common practice in this area. Trees may be interplanted within cropland as alleys or along boundaries (Owate, 2018). In addition, intercropping trees with cereals and legumes as mixture is more profitable and beneficial to the biological functioning of the ecosystem with the advantages of suppressed incidence of weeds, efficient use of available resources thereby increasing yields and most importantly, providing biological and economic stability (Lienhard *et al.*, 2020) to the ecosystem. Five *G. robusta* tree seedlings, spaced at 2 m between seedlings, were planted in a row along the width of each plot at a point 5 m from one edge of the plot width and 10 m from the opposite edge. A transect line perpendicular to the row of *G. robusta* seedlings was established at the centre along the length of each plot. Three cropping zones, A, B and C, were established adjacent to each other on the 10 m side of the plots starting at a point 0.5 m from the *G. robusta* seedling rows. Using a soil corer of 7.5 cm diameter and 5 cm depth, baseline soil samples were taken at a depth of 0-10

cm from five randomly selected spots within each cropping zone and the samples treated as in section 3.3.1 above. Two grams of *M. anisopliae* granules were applied to each planted *G. robusta* seedling in each plot, as described in section 3.4.3 above. Three different cropping regimes; (i) sole maize, (ii) maize + groundnut intercrop and (iii) fallow were randomly allocated to the plots and the crops planted in their respective plots.

3.4.1 Horizontal spread of *M. anisopliae* in the soil

At 12 and 24 weeks after application of *M. anisopliae* granules to *G. robusta* seedlings in rows, subsequent soil sampling, after the baseline in section 3.5 above, was done at five randomly selected spots in each cropping zone of every plot. With the aid of a 0.5 square metre wooden frame with eight quadrats (Figure 3.7) placed at every spot, soil samples were taken at a depth of 0-15 cm from six randomly selected quadrats using a 2 cm diameter soil auger. The samples from the quadrats were aggregated to form one composite soil sample from one spot. The soil composites were then bulked to form one representative soil sample for each cropping zone, packed in transparent polythene bags (KPL) and the bags with their contents put in a cooler box for transportation to the pathology laboratory at SFEGS, Makerere University. In the laboratory, approximately 250 g sub samples were removed from each representative sample and placed in sterile 2 L plastic buckets (KPL) and treated as described in section 3.3.2 above. After every sampling from a cropping zone, the soil auger was surface sterilized by wiping using cotton wool soaked in 96% ethanol before sampling was done in another cropping zone.

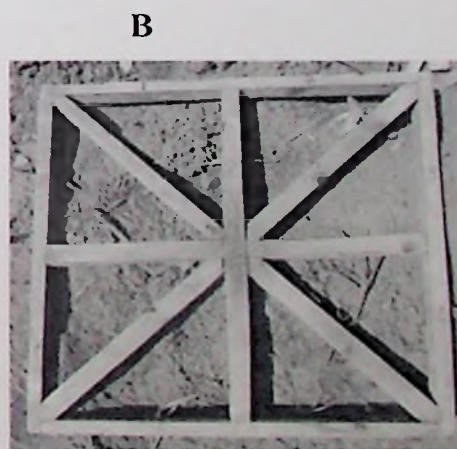
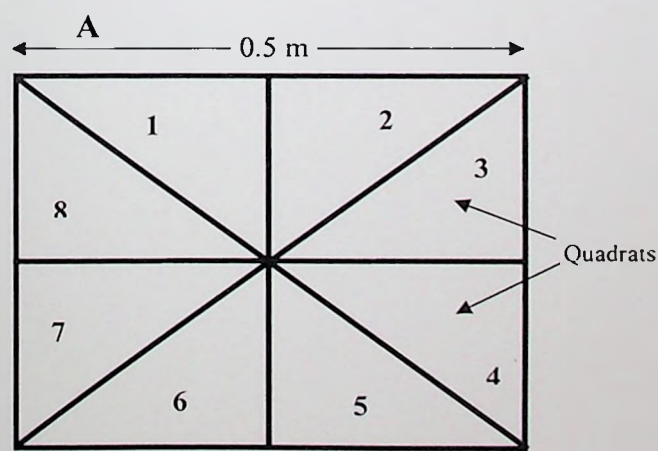


Figure 3.7: Sketch diagramme (A); and Photograph of wooden frame (B) used in soil sampling

3.4.2 Vertical spread of *M. anisopliae* in the soil

At 44 weeks after application of *M. anisopliae* granules to *G. robusta*, soil samples were taken at a distance of 8 cm from the *G. robusta* stem within the root zone of two randomly selected

G. robusta tree seedlings in each plot. Sampling was done at depths of 0 cm – 10 cm and 10 cm – 20 cm using the same soil auger described in section 3.5.1 above. After sampling at each depth, the soil auger was washed with borehole water, dried using a dry clean rag and surface sterilized using cotton wool soaked in 96% ethanol. The soil samples were separately put in transparent polythene bags (KPL, Nairobi) and placed in a cooler box for transportation to the pathology laboratory at SFEGS, Makerere University. In the laboratory, the samples were bulked according to their sampling depths, thoroughly mixed while breaking any soil clods to form a homogenous sample. From each bulked soil sample, approximately 250 g sub samples were removed and placed in sterile 2 L plastic buckets (KPL) and again treated as described in section 3.3.2 above.

3.5 Effect of *M. anisopliae* on non-target ground arthropods

Trapping of arthropod specimens was initially done prior (0 WAP) to application of *M. anisopliae* granules to *Grevillea* seedlings as described in section 3.5 above. This was done by inserting two plastic bucket pitfall traps of 16 cm depth and 14.5 cm diameter to the ground to level with the ground surface (Abonyo *et al.*, 2016), at distances of 3 m and 6 m from the row of *G. robusta* along the transect lines at the centre of each plot (**Figure 3.8**). The plastic bucket covers were removed and the buckets left open overnight to trap any ground crawling arthropods. The following morning, the bucket pitfall traps were inspected and any containing trapped arthropod specimens inside them had their covers replaced and recovered from the ground. The recovered buckets, with their contents were transported to Makerere University, SFECS pathology laboratory. In the laboratory, the arthropods were removed from the buckets immediately after arrival using a pair of forceps sterilized by wiping with cotton wool soaked in 96% ethanol and placed in sterile plastic petri dishes lined with filter paper moistened with distilled water (Mathulwe, 2019). The petri dishes with their contents were incubated at room temperature on a lamina floor sterilized with 96% ethanol and continuously inspected at intervals of two days. Any arthropod cadavers that showed a green growth of fungal spores characteristic of *M. anisopliae* were removed from the petri dish using a sterile pair of forceps, counted and recorded. Subsequent trapping of arthropods was done at 12 and 28 weeks after application (WAP) of *M. anisopliae* granules to the *G. robusta* tree seedlings. The subsequently trapped arthropod specimens were as well transported to Makerere University, SFECS pathology laboratory, and treated as described as before.

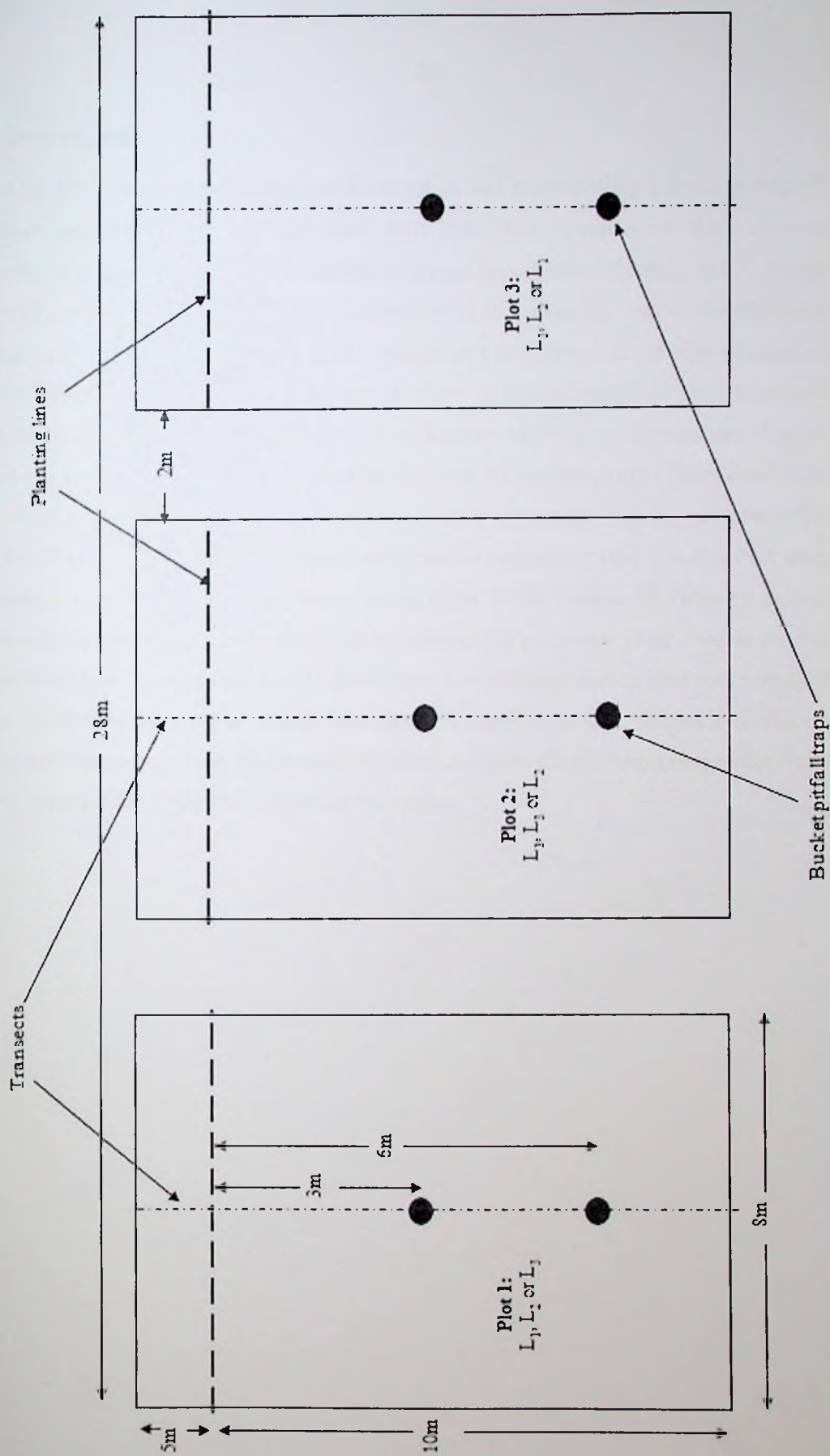


Figure 3.9: Sketch diagram of the plot lay-out for trapping of non-target ground arthropods

3.6 Data analysis

Data on *M. anisopliae* persistence and spread in soil were obtained from counts of termite cadaver specimens removed from each petri dish. The counts were then expressed as a proportion of those that showed incidence of fungal spores after exposure to soil samples from the study sites. Data on optimum application rate of *M. anisopliae* was obtained from monthly counts of *G. robusta* seedling survival expressed as a proportion of the total seedlings per plot for each treatment applied; whereas data on effect of *M. anisopliae* on non-target arthropods was obtained from counts of incidence of arthropod cadaver specimens showing the green spore growth of *M. anisopliae* after incubation at each trapping phase. These data were as well expressed as a proportion of total arthropod cadavers incubated. The derived proportions were expressed as percentages and these were subjected to analysis of variance ANOVA using GLM procedure at 95% level of significance using IBM SPSS version 20 (Murigu *et al.*, 2016). Further analysis on persistence was done by conducting an independent *t*-test to determine the mean differences in persistence of *M. anisopliae* between application sites and non-application sites. Analysis of data on *M. anisopliae* optimum application rate, spread and effect on non-target arthropods was further performed by conducting Post hoc comparison using Tukey HSD test to separate the differences between the means.

CHAPTER 4

RESULTS

4.1 Persistence of *M. anisopliae* in soil

M. anisopliae was found to be persistent at all farm application sites in the three locations of Namulonge, Ikulwe and Namukubembe. The cassava-maize mixed cropping activity exhibited the highest persistence across all sites i.e. Namulonge (86.7%), Namukubembe (83.3%) and Ikulwe (76.7%) whereas the fallow revealed the lowest levels of persistence with Namulonge showing 70.0% and Ikulwe with 63.3% (Table 4.1). A univariate GLM ANOVA in IBM SPSS version 20 indicated no significant effects of farm location ($F_{(2, 28)} = 0.793$, $p = 0.462$) and farming activity ($F_{(3, 28)} = 0.972$, $p = 0.420$) on persistence of *M. anisopliae* in the soil. Application of *M. anisopliae* had a significant effect ($F_{(1, 28)} = 276.391$, $p < 0.001$) on persistence at the different site activities in the different site locations. There was a non-significant interaction effect between farm location, farming activities and *M. anisopliae* application ($F_{(7, 28)} = 0.562$, $p = 0.780$) on soil persistence of the fungus. Mean persistence of *M. anisopliae* at sites of application ($M = 75.2\%$, $S.D = 12.89$) was significantly higher ($t_{(40)} = 16.69$, $p < 0.001$) than at sites where the fungus was not applied ($M = 11.9\%$, $S.D = 11.67$).

Table 4.1: Persistence of *M. anisopliae* in soil at different farm activities

Farming activity	Mean persistence (%)		<i>p</i> - value
	<i>M. anisopliae</i> applied	No application	
Namulonge			
Tilled without crop	76.7	13.3	0.003
Cassava + Maize	86.7	6.7	0.001
Grass fallow	70.0	6.7	0.001
Ikulwe			
Cassava + Maize	76.7	13.3	0.001
Grass fallow	63.3	10.0	0.016
<i>G. robusta</i> tree stand	70.0	10.0	0.007
Namukubembe			
Cassava + Maize	83.3	23.3	0.006
Mean ± <i>S.D</i>	75.2 ± 12.89	11.9 ± 11.67	< 0.001

4.2 Optimum application dose of granular and oil-based *M. anisopliae* formulations

4.2.1 Survival pattern of *G. robusta*

There was an overall decline of between 27.5% and 46.7% on survival of *G. robusta* seedlings exhibited across all treatments over ten months (June 2011 – April 2012) after planting (Figure 4.1). The *M. anisopliae* treatment applications exhibited a combined *G. robusta* seedling survival decline of 36.7% over the first seven months (June 2011 to December 2011) after planting whereas the control treatment alone registered a decline in seedling survival of 46.7% during the same period. In the period December 2011 to April; 2012, there was a significant increase in seedling survival with the treatment applications registering an average mortality of only 8.3% in comparison to the control treatment which, alone, recorded a further seedling survival reduction of 17.5% during the same period. Survival rate of *G. robusta* seedlings for the different doses of *M. anisopliae* was significantly different ($F_{(10, 330)} = 420.6, p < 0.001$) over the ten months period.

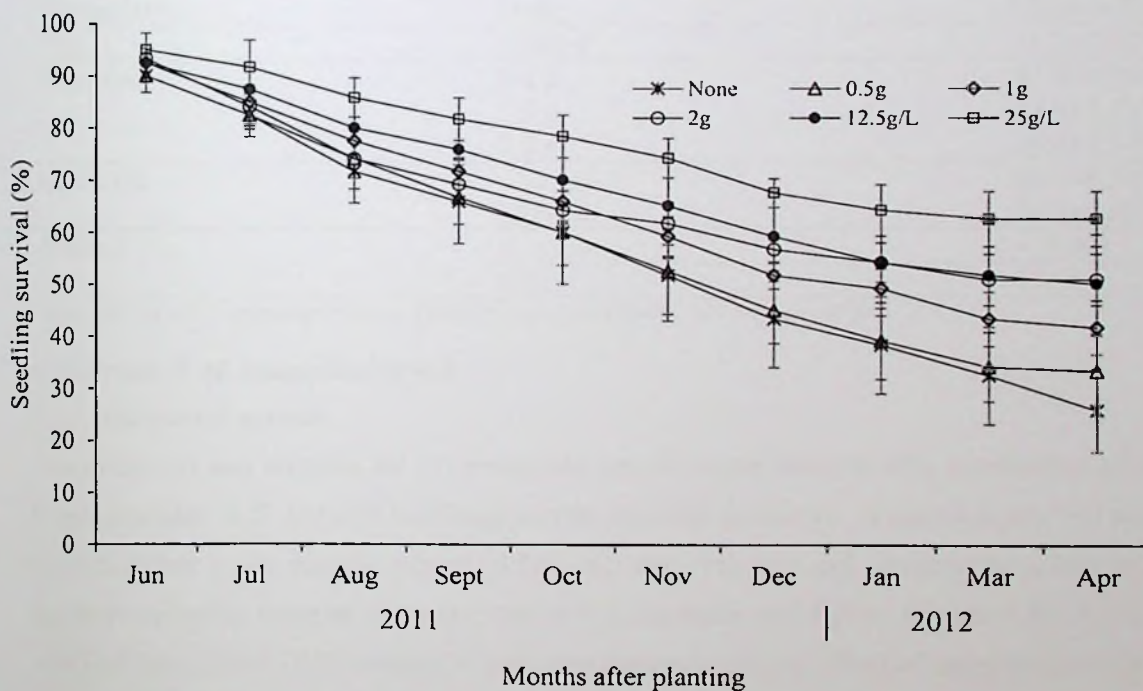


Figure 4.1: Survival trends of *G. robusta* seedlings over 11 months after *M. anisopliae* application at planting

4.2.2 *G. robusta* seedling survival at varying *M. anisopliae* doses

A one way ANOVA revealed significant differences in the effect of the different treatments ($F_{(5, 30)} = 13.77, p < 0.001$) on mean survival of *G. robusta* seedlings (Table 4.2). Post hoc comparisons with Tukey HSD test revealed the highest mean seedling survival ($76.3 \pm 2.62\%$)

for the 25 g/L dose of the aqueous formulation compared to the 2g ($66.4 \pm 2.56\%$), ($p = 0.010$); 1g (63.9 ± 2.52), ($p = 0.001$) and 0.5 g (57.8 ± 4.76), ($p < 0.001$) doses of the granular formulation. Mean seedling survivals ($76.3 \pm 2.62\%$ and $68.8 \pm 6.51\%$) between the higher dose (25 g/L) of the oil-based formulation and half dose (12.5 g/L) of the same formulation were not significantly different, ($p = 0.095$). There was also a significant difference in mean seedling survival ($57.8 \pm 4.76\%$) between the 0.5 g dose ($p = 0.007$) of the granules and the 12.5 g/L dose of the aqueous formulation with mean survival of $68.8 \pm 6.51\%$. The non-application (control) treatment ($56.2 \pm 7.32\%$) was significantly different from the 2g dose ($66.4 \pm 2.56\%$) of the granules ($p = 0.019$) and both of the 12.5 g/L ($p = 0.002$); and the 25 g/L ($p < 0.001$) doses of the aqueous solutions. There was also no significant difference between the non-application treatment and the granular formulations of 1g ($p = 0.108$) and 0.5 g ($p = 0.993$) doses.

Table 4.2 Mean survival (%) of *G. robusta* tree seedlings under varying doses of *M. anisopliae* formulations

Formulation	Dose	Mean $\pm$ S.D
Non	Control	56.2 ± 7.32^a
Granules	0.5 g	57.8 ± 4.76^a
	1.0 g	63.9 ± 2.52^{abc}
	2.0 g	66.4 ± 2.56^{bc}
Oil-based	12.5 g/L	68.8 ± 6.51^{cd}
	25.0g/L	76.3 ± 2.62^d
Statistic	<i>p</i> - value	< 0.001
	<i>S.E</i>	1.995

Values with the same superscript letters in a column are not significantly different ($p = 0.05$)

4.3 Spread of *M. anisopliae* in soil

4.3.1 Horizontal spread

Assessment of soil samples for *M. anisopliae* spread eleven months after application of the fungal granules to *G. robusta* seedlings in rows revealed incidence of spores in the soil at all three cropping zones namely nearest (0.5m-1m); mid (1m-5m) and furthest (5m-10m) in all the three cropping regimes of maize, maize + groundnuts and fallow (Table 4.3). A GLM ANOVA using IBM SPSS version 20 indicated a non-significant effect of cropping zone ($F_{(2, 45)} = 0.45$, $p = 0.638$) on *M. anisopliae* incidence whereas cropping regime had a significant effect ($F_{(2, 45)} = 10.69$, $p < 0.001$) on incidence of the fungal spores in the soil. The interaction effect between cropping regime and sampling zone on incidence of *M. anisopliae* in soil was non-significant, ($F_{(4, 45)} = 1.06$, $p = 0.387$).

Table 4.3: Mean incidence (%) of *M. anisopliae* under different cropping regimes

Cropping regime	Cropping Zone			Mean $\pm$ S.D
	Zone A (0.5 – 1.5 m)	Zone B (1.5 – 3.0m)	Zone C (3.0 – 5.0m)	
Maize	11.0 ^a	14.2 ^a	10.7 ^a	11.9 $\pm$ 7.34 ^a
Maize + Ground nuts	26.5 ^b	20.2 ^a	17.7 ^a	21.5 $\pm$ 8.27 ^b
Fallow (Control)	8.1 ^a	11.1 ^a	10.6 ^a	9.9 $\pm$ 8.10 ^a
Mean $\pm$ S.D	15.2 $\pm$ 10.37	15.1 $\pm$ 9.66	13.0 $\pm$ 9.97	14.45 $\pm$ 9.27
<i>p</i> - value	< 0.001	0.267	0.223	< 0.001

Values with the same superscript letters in a column are not significantly different ($p = 0.05$)

Mean incidence across cropping regimes (14.45 $\pm$ 9.27%) in all the cropping zones was significantly different ($p < 0.001$) with the highest seen under maize + groundnut intercrop (21.5 $\pm$ 8.27%) compared to sole maize and fallow which had spore incidences of 11.9 $\pm$ 7.34% and 9.9 $\pm$ 8.10% respectively. At the nearest cropping zone, mean incidence of *M. anisopliae* (15.2 $\pm$ 10.37%) was significantly different ($p < 0.001$) across cropping regimes whereas mean incidence in the mid (15.1 $\pm$ 9.66%) and furthest (13.0 $\pm$ 9.97%) cropping zones were non-significant, ($p = 0.267$); ($p = 0.223$) respectively. Post hoc comparisons with Tukey HSD test revealed that in the nearest cropping zone, incidence under the intercrop regime (26.5%) was significantly higher ($p = 0.003$) than incidence under maize cropping regime (11.9%); whereas incidence under fallow regime (9.9%) was non-significantly lower ($p = 0.735$) than incidence under sole maize cropping regime in the same cropping zone. At the mid cropping zone, the maize + groundnut intercrop regime showed higher *M. anisopliae* incidence of 20.2% than the sole maize regime with 14.2% which was statistically non-significant ($p = 0.528$) and was also higher than incidence under the fallow regime (11.1%) which was also statistically non-significant, ($p = 0.838$). Although *M. anisopliae* incidence under sole maize (14.2%) was higher than fallow (11.1%) cropping regime, the difference was statistically non-significant ($p = 0.248$). The same trend was observed at the furthest cropping zone where *M. anisopliae* incidence under the intercrop regime (17.7%) was higher than both sole maize (10.7%) and fallow (10.6%), the differences were statistically non-significant, ($p = 0.286$) and ($p = 1.000$) respectively. Incidence under sole maize (10.7%) and fallow (10.6%) regimes were comparably non-significant ($p = 0.283$) at the same cropping zone.

4.3.2 Vertical spread

M. anisopliae was found to spread vertically in the soil horizons with incidence higher in upper horizon than the lower horizon (Table 4.4). A paired samples *t*-test indicated that incidence of the fungal spores was higher in the upper soil layer ($M = 16.9\%$, $S.D = 14.89$) than in the deeper

soil layer ($M = 11.7\%$, $S.D = 14.44$), the difference was non-significant ($t_{(35)} = 1.424$, $p = 0.163$). A one way ANOVA indicated a significant effect of cropping regime ($F_{(2, 33)} = 3.542$, $p = 0.040$) on *M. anisopliae* incidence at the upper soil zone whereas in the lower soil zone cropping regime had a non-significant effect ($F_{(2, 33)} = 0.152$, $p = 0.860$) on *M. anisopliae* incidence.

Table 4.4 Mean incidence (%) of *M. anisopliae* at different soil layers

Cropping regime	Soil Zone	
	Upper (0-10) cm	Lower (10-20) cm
Maize	8.3 ^a	10.0 ^a
Maize + Groundnuts	22.5 ^b	11.7 ^a
Fallow	20.0 ^{ab}	13.3 ^a
Mean $\pm$ S.D	16.9 $\pm$ 14.89%	11.7 $\pm$ 14.44%
<i>p</i> = value	0.040	0.860

Values with the same superscript letters in a column are not significantly different ($p = 0.05$)

Post hoc comparisons with Tukey HSD test revealed that at the upper soil zone, incidence under the intercrop regime (22.5%) was significantly higher ($p = 0.046$) than incidence under sole maize cropping regime (8.3%); whereas incidence under fallow regime (20.0%) was non-significantly higher ($p = 0.115$) than incidence under sole maize cropping regime in the same soil zone. At the lower soil zone, the fallow cropping regime showed higher *M. anisopliae* incidence of 13.3% than either sole maize regime with 10.0% or maize + groundnut inter crop regime (11.7%) which were both statistically non-significant; ($p = 0.846$) and ($p = 0.959$) respectively. Incidence under sole maize regime (10.0%) was marginally higher than incidence under maize + groundnut inter crop regime and the difference was statistically non-significant, ($p = 0.959$).

4.4 Effect of *M. anisopliae* on non-target arthropods

There were 89 individuals of non-target arthropods of the orders *Hymenoptera*, *Dermaptera*, *Coleoptera*, *Orthoptera* and *Araneae* that were trapped in the 3m area whereas 61 specimens of the same orders were trapped in the 6m area in all the three (0 WAP, 12 WAP and 28 WAP) trapping phases (Table 4.5). There was a non-significant effect of *M. anisopliae* infection at different trapping phases of non-target arthropods in the 3m ($F_{(2, 89)} = 0.559$, $p = 0.574$) and 6m ($F_{(2, 61)} = 1.216$, $p = 0.306$) areas. There was also a non-significant effect of *M. anisopliae* infection on non-target arthropod order in the 3m ($F_{(4, 89)} = 1.137$, $p = 0.346$) and 6m ($F_{(4, 61)} = 2.031$, $p = 0.106$) trapping areas. The interaction effect of trapping phase and non-target

arthropod order was non-significant in the 3m ($F_{(8, 89)} = 0.800, p = 0.604$) and 6m ($F_{(8, 61)} = 1.091, p = 0.387$) trapping areas.

Table 4.5: Effect of *M. anisopliae* on non-target arthropods

Arthropod Order	N	Mean (%) arthropod not infected			Mean $\pm$ <i>SD</i>	<i>p</i> - value
		0 WAP	12 WAP	28 WAP		
3 m						
<i>Hymenoptera</i>	18	100.0	93.3	100.0	98.1 $\pm$ 7.86 ^a	0.288
<i>Dermaptera</i>	21	100.0	100.0	96.4	98.8 $\pm$ 5.46 ^a	0.387
<i>Coleoptera</i>	13	100.0	100.0	95.8	98.1 $\pm$ 6.93 ^a	0.600
<i>Orthoptera</i>	15	89.3	87.1	100.0	90.4 $\pm$ 17.41 ^a	0.591
<i>Araneae</i>	22	100.0	96.4	95.8	97.7 $\pm$ 7.36 ^a	0.501
6 m						
<i>Hymenoptera</i>	14	100.0	95.0	100.0	98.2 $\pm$ 6.68 ^a	0.441
<i>Dermaptera</i>	9	83.3	100.0	85.0	88.0 $\pm$ 19.14 ^a	0.662
<i>Coleoptera</i>	18	88.9	95.2	100.0	96.3 $\pm$ 10.76 ^a	0.315
<i>Orthoptera</i>	9	83.3	83.3	80.4	82.0 $\pm$ 18.85 ^a	0.980
<i>Araneae</i>	11	90.0	100.0	72.2	87.9 $\pm$ 18.01 ^a	0.158

WAP=Weeks After *M. anisopliae* Application;

Values with the same superscript letter in a row are not significantly different ($p = 0.05$)

An independent *t*-test revealed that the proportion of individual non-target arthropod cadavers not infected by *M. anisopliae* in the 3m area was higher ($M = 96.9\%$, $S.D = 9.75$) than non-infection in the 6m ($M = 91.9\%$, $S.D = 15.10$) area in all the three trapping phases (0 WAP, 12 WAP, 28 WAP) and the difference was statistically non-significant ($t_{(94)} = 2.28, p = 0.25$). Among the non-target arthropod specimens trapped in the 3m area during the three trapping phases, *Hymenoptera* had none of its individuals (100%) infected in the pre-application (0 WAP) and third (28 WAP) trapping phases whereas the second trapping phase (12 WAP) had the least non-infection (93.3%) of non-target arthropods. Both *Dermaptera* and *Coleoptera* had none of their individuals infected by *M. anisopliae* in the first and second trapping phases whereas in the third trapping phase, *Dermaptera* had 96.4% while *Coleoptera* had 95.8% of their individuals not infected by the fungus in the 3m area. In the first and second trapping phases, *Orthoptera* had 89.3% and 87.1% non-infections, respectively, while no infection of its individuals was recorded in the third trapping phase. For *Araneae*, 100% non-infection of its cadavers was recorded in the pre-application trapping phase while the second and third trapping phases, respectively, saw 96.4% and 95.8% of its individuals not infected by the *M. anisopliae*. In the 6m arthropod trapping zone, again *Hymenoptera* had 100% of its individuals not infected by *M. anisopliae* in the pre-application and third trapping phases whereas 95% non-infection was recorded in the second trapping phase. With *Dermaptera*, 100% non-infection of its individuals was seen in the second trapping phase whereas the first and third trapping phases recorded *M. anisopliae* non-infections of 83.3% and 85.0% respectively.

CHAPTER 5

DISCUSSION OF RESULTS

5.1 Persistence of *M. anisopliae* in the soil

The significantly higher persistence of *M. anisopliae* fungal spores in areas of application (75.4%) compared to areas of non-application (11.9%), as found in this study, suggests that *M. anisopliae* is an effective bio control agent against pestiferous termites of economic importance in agriculture. This demonstrates that there is no need for repeated application for at least a decade hence offering an effective method of termite pest control. A study by Castro *et al.* (2016) demonstrated that applied isolates of *Metarhizium* haploids were often recovered in their specific treatment plots for up to one year after application, but they were also found in un-inoculated soil from control plots in the fields, a finding which is consistent with that of the current study. This may also suggest that there are naturally existing strains of *Metarhizium* fungal spores in the study sites that need to be understood and effectiveness in pest control as well needs to be understood. Further still, the higher persistence in the mixed cropping practice compared to sole cropping practice across all the study sites as shown in the results (Table 4.1) points to some changes within the soil as a result of the interaction between the fungal spores with growing crops. Uptake of carbon exudated into the soil around the rhizosphere by the developing crop root system might be responsible for sustaining survival of *Metarhizium* fungal populations in cropped land (Liu *et al.*, 2016). In a study by Yang *et al.* (2018), it was shown that persistence of *M. anisopliae* in the soil of a corn field while remaining viable lasted for up to 15 months. This is in line with the findings of my study where *M. anisopliae* persistence was high especially in cropped areas where there were favourable conditions that may have enhanced survival and proliferation of fungal populations for over a decade (2000-2012) after application. The ability of *M. anisopliae*, ICIPE 30, to maintain adequately viable populations in the soil is useful property in managing the incidence of pestiferous insect organisms in agriculture and forestry. This is in line with the findings of Yang *et al.* (2018), which showed that persistence and efficacy of a strain of *M. anisopliae*, Ma09, were not altered under field conditions throughout the 360 days of the study.

5.2 Optimum application rate of *M. anisopliae*

It was clearly evident that the higher doses of both the granular (2g per seedling) and oil-based (25g/L per seedling) formulations *M. anisopliae* applications caused higher survival rates of

G. robusta seedlings over ten months whereas the non-application treatment had the lowest survival rate over the same period. Optimum seedling survivals of $76.3 \pm 2.62\%$ and $66.4 \pm 2.56\%$ for the higher doses of the oil-based (25/L) and granular (2g) formulations, respectively, applied per seedling suggest that increasing the dose of the fungus renders it more effective in protecting *Grevillea* seedlings against termite attack thus increased seedling survival of over 60% when applied at planting under field conditions. Lowering the application dose has a corresponding effect of reduced seedling survival, however, the lowest seedling survival is shown by the non-application (control) treatment which registered only $56.2 \pm 7.32\%$ mean seedling survival. In addition to demonstrating the potential of *M. anisopliae* as a potential bio-agent for control of pests of economic importance in forestry and agriculture, as has been shown in studies by (Sahayaraj, 2018) and (Qiu *et al.*, 2019), it is critical to highlight appropriate dose levels for field application in termite pest control. Although some studies have been carried out to determine effective doses and or concentrations of *Metarhizium* for insect pest management, these have largely been under laboratory control. For example, Remadevi *et al.* (2010) demonstrated that different isolates of *M. anisopliae* with high lethal concentrations resulted in high mortality of *Odontotermes* spp. under laboratory conditions. In a study to identify potential virulent isolates of *Metarhizium* spp. for management of pestiferous termites, it was demonstrated that fungal isolates could be formulated with either non-repellent or attractant components to enhance their efficacy in insect pest control (Ravindran *et al.*, 2015). The findings of this study highlight the importance of developing prescribed doses of *M. anisopliae* bio-agent for field application, the same way it has been demonstrated under laboratory conditions. Effective deterrence to pestiferous insects of economic importance in agriculture is dependent upon the effectiveness of a bio-control method in causing virulent epizootics. To enhance fungal conidial attachment to the insect cuticle and subsequent germination on the cuticle (Brunner-Mendoza *et al.*, 2019), it is key, among other factors, that the dose of the fungal spores is known and adequate enough to cause virulence in the insect pest. This agrees with the findings of this study where significantly higher seedling survival ($76.3 \pm 2.62\%$) was seen with the 25 g/L dose of oil-based formulation compared to the higher dose ($66.4 \pm 2.56\%$) of the 2g granular formulation, ($p = 0.010$). A study by Indriyanti *et al.* (2017) demonstrated that there was a positive correlation between *Metarhizium* dose and mortality of *O. rhinoceros* after 8 weeks, where high mortality was registered at the larval stage of the insect. Our study also demonstrates that formulation of the entomopathogen to the target is a key factor. The aqueous spore formulation with vegetable oil caused higher survival rates of *G. robusta* tree seedling as compared to the granulated

formulation under field conditions although the difference was not significant. In a study by (Batta and Kavallieratos, 2018), formulated strains of entomopathogenic fungi *B. bassiana* and *M. anisopliae* Sorokin (Ascomycota: Hypocreales) against stored grain insects have been proven more effective with adult mortality ranging from 79% to 95% on the target pest insect species thus conforming with the findings of this study.

5.3 Spread and penetration of *M. anisopliae* in the soil

Horizontal spread of *M. anisopliae* fungal spores in soil under each of the three cropping regimes (maize; maize + groundnut, fallow), was significant ($F_{(2, 45)} = 10.69, p < 0.001$) with higher mean spread exhibited under maize + ground nut intercrop ($21.5 \pm 8.27\%$) compared to sole maize ($11.9 \pm 7.34\%$) and the fallow ($9.9 \pm 8.10\%$) regimes irrespective of the three cropping zone (0.5 – 1.5 m, 1.5 – 3.0m and 3.0 – 5.0m) relative to the point of fungal inoculation. This could have been due to the high concentration of conidial carrier agents that may have been attracted by a variety of food material that may have been offered by the maize and groundnut crops thus resulting in higher concentration of fungal conidia in this region. Disaggregated cropping zone data also revealed more spread at the nearest (0.5m – 1.0m) cropping zone to the fungal inoculation point for the intercrop regime (26.5%) compared to sole maize (11.0%) and fallow (8.1%) cropping regimes in the same cropping zone. This finding suggests that the higher concentration of *M. anisopliae* conidia in the region closer to the point application under maize + ground nut intercrop is due to the favourable bio-chemical factors such as rhizospheric carbon, nitrogen, soil enzyme activity, pH, redox potential that prevailed in the surrounding soil in the vicinity of the applied *M. anisopliae*, hence allowing the surviving spores to easily adapt and multiply. This could be due to the fact that entomopathogenic fungi are capable of utilising free rhizospheric carbon and positively interact with the plant root system to maintain survival and growth (Liu *et al.*, 2016). For the cropping zones distant (1.0m – 5.0m) and 5.0m – 10.0m) from the *Grevillea* root system, soil factors must have changed less to the disadvantage of the *M. anisopliae* spores in these areas. There could also be contention that agronomic practices like weeding could have played a role in the dispersal of fungal conidia from the point of application of the fungal granules. In the fallow regime where there is no soil disturbance, less of the fungal conidial concentrations are expected in this area. This is in line with findings by Castro *et al.* (2016) in which their isolation studies of pathogenic fungi in Brazil noted that agronomic manipulations play a contributory role in the dispersal of fungal isolates, thus supporting our opinion in this study. In addition,

the effect of rhizospheric root competition by the fungus around *G. robusta* trees inoculation points may have played a role in sustaining survival of the fungal spores around the root zone. This study also revealed that *M. anisopliae* spread in relation to soil depth was significant with higher ($16.9 \pm 14.89\%$) spread of fungal spores at the upper (0 – 10cm) soil zone than spread ($11.7 \pm 14.44\%$) at the lower (20 – 20cm) soil layers, $p = 0.040$. This is in agreement with the findings of Liu *et al.* (2016) where they revealed that survival of *M. anisopliae* at the 3 – 5 cm soil depth was higher than at the lower depth of 13 – 15 cm. Various factors influence survival of *Metarhizium* in the soil including ultra violet light, inorganic chemicals, soil type, temperature, humidity and agricultural practice (Yang *et al.*, 2018).

5.4 Effect of *M. anisopliae* on non-target arthropods

Results show no *M. anisopliae* mycosis on non-target arthropod orders trapped at the different phases in the different areas. There was a non-significant effect of *M. anisopliae* infection at 0, 12 and 28 Weeks after Application of fungal granules in the 3m ($F_{(2, 89)} = 0.559, p = 0.574$) and 6m ($F_{(2, 61)} = 1.216, p = 0.306$) areas. There was also a non-significant effect of *M. anisopliae* infection on non-target arthropod order in the 3m ($F_{(4, 89)} = 1.137, p = 0.346$) and 6m ($F_{(4, 61)} = 2.031, p = 0.106$) trapping areas. *M. anisopliae* does not affect non-target arthropods when it is applied in soil for control of pest termites. Non-target organisms as part of the biotic factors in the environment may play a key role in the efficacy of a bio-control agent, for example, in disseminating the agent to affected areas. Therefore, survival and behaviour of non-targeted organisms upon exposure to a bio-control agent is an important attribute in insect pest control using entomopathogens. In line with my study findings, an investigation by (Abonyo *et al.*, 2016), using laboratory bioassays and field experimentation did not find evidence of harmful effects of *M. anisopliae* on ants usually associated with *Odontotermes* sp. termitaria in Kenya. Further still in an experiment using *M. brunneum* EAMa 01/58-Su strain for managing olive fruit fly in olive orchards, it was noted that soil dwelling arthropods were not affected by application of the fungus both in the field and greenhouse conditions (Yousef *et al.*, 2017). The findings from this study also suggest that even at a distance away from the point of application there is traceable evidence of fungal spore presence among beetles (*Coleoptera*) and earwigs (*Dermaptera*) which may have probably been carried to this point by these insects.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusion

In broad terms, this study has shown that *M. anisopliae* can be used as an integral part of the termite pest management efforts under farm conditions in Uganda. The ability of the fungal spores to remain active in the soil for a prolonged period of time; ability to improve survival of *G. robusta* tree seedlings under termite infestation; spread of the fungal into areas surrounding the points of application; and its negligibly detectable effect on other insect organisms not targeted within the application areas. These findings agree with those of other research studies that have delved into integrating the fungus as a component of bio control agents that could be developed and used in agriculture and forestry for the management of pests of economic importance in Uganda. Below are concluding highlights based on the findings from this study:

- *M. anisopliae* can persist in the soil for over a decade and incidence of the fungal spores is higher in farm sites under mixed cropping than those under sole cropping.
- The highest dose (25g/L per seedling) of the oil-based formulation showed the highest seedling survival of 76.3% hence the lowest incidence of termites whereas the non-application treatment/control had the highest termite incidence with seedling survival of only 56.2% over ten months of the study
- *M. anisopliae* does not spread in the soil horizontally within a distance of 5.0 m from the site of application of fungal granules. However marginal fungal incidence can be detected within a distance of 2.0 m from the spot of application of fungal granules in the mixed cropping regime. At any spot beyond 2.0 m, no incidence of *M. anisopliae* can be detected either in the sole, mixed cropping or fallow cropping regimes in the ten months of the study.
- Exposure of non-target arthropods to *M. anisopliae* has no effect on the organisms with high proportions of over 93% of non-infections noted up to a distance of 6 m from the point of application of fungal granules.
- Soil disturbance through land preparation, for example, initial land tilling and weeding might be vital in achieving dispersal of the fungal spore from spots of application to areas where it might be useful in achieving pest control. This is evidenced by the fact that it could not be detected in areas distant from the point of application and at lower soil profiles.

- Non-target ground arthropods not affected by *M. anisopliae* are potentially useful in spreading the fungus to further away areas in addition to agronomic practices like weeding.

6.2 Recommendations

- There is need to assess possible natural occurrence of any strains of *Metarhizium* that maybe present under prevailing natural conditions since there was some traceable presence of the fungus in non-application sites.
- Rhizospheric competition of *M. anisopliae* in different cropping systems should be investigated to understand its high proliferation and persistence under inter-cropping regime.
- There is need to evaluate *M. anisopliae* for its effectiveness in termite control under different agro ecological zones of Uganda in order to determine its suitability in a wider geographical scope.

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